Abstract citation ID: gfae069.130

#2086
Understanding B cell memory autoreactivity in idiopathic membranous nephropathy for tailored therapy

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Background and Aims: Idiopathic membranous nephropathy (iMN) is an autoantibody-mediated glomerular disease, and one of the leading causes of nephrotic syndrome in adults. iMN is caused by the formation of immune complexes composed by IgG autoantibodies and target antigens expressed on podocytes. The main autoantigen is the phospholipase A₂ receptor1 (PLA2R1) and the antibodies are mainly belonging to the IgG4 isotype. Those immune complexes are responsible for profound changes in the structure and function of the podocytes and disruption of the glomerular barrier. Nevertheless, despite complement components at the site of glomerular injury have been found in biopsies from patients with MN, many factors involved in the pathogenesis are still unclear. We overall aim at characterizing both the serological and the memory cellular component in iMN patients, with identification of dominant B cell epitopes recognized by circulating autoantibodies and autoreactive memory B cells, which could be potentially used to deplete PLA2R1 specific memory B cells and plasma blasts.

Method: We analyzed the serum from a cohort of 159 iMN patients collected at diagnosis without any previous or concomitant treatment as well as a similar number of healthy controls’ samples. An ELISA-based quantification method has been used to estimate the concentration of circulating anti-PLA2R1 antibodies. Furthermore, we processed 27 additional blood samples from independent iMN patients as well as age and gender matched healthy controls to extract and analyze peripheral blood mononuclear cells by flow cytometry (BD FACS Fortessa) and identify the major B and T cell populations via multi-color staining. In order to identify and clone human anti-PLA2R1 antibodies from primary memory B cells we reproduced the experimental workflow exactly as described in Lindner J.M. et al. Immunity 2019. The isolated antibodies were then characterized with analytical methods (affinity, binding, epitope determination and complement activation ability) and used in competition assays.

Results: A deep analysis of the serum reactivity has been performed and iMN patients showed a high prevalence of autoantibodies against PLA2R1, mostly of high affine IgG4 subclass with a broad concentration range (1-20ug/ml). A concomitant IgG1 response versus PLA2R1 has been also detected in the serum in different proportion with IgG4. An extended immunophenotyping of circulating peripheral blood mononuclear cells revealed an altered B and T cell compartment in iMN patients. In particular, we described an expansion of circulating CD27⁺ IgG4 memory B cells, IgG4 plasma blasts, CD4+ T follicular helper cells and CX3CR1⁺ T peripheral helper cells. Moreover, we isolated and cloned five very specific, highly affine, and somatically hypermutated patient’s derived anti-PLA2R1 antibodies. Competition experiments between patient sera and patients’ derived monoclonal antibodies showed a strong inhibitory effect, indicating the presence of a shared epitope recognized on the surface of the CysR domain of PLA2R1. The identified epitope has also shown an important role in complement activation, despite a component patient-specific seems to be involved as well.

Conclusion: Overall, we performed a comprehensive characterization of the autoreactive B cell memory compartment in iMN, shedding lights on the complexity of the autoreactive repertoire. Indeed, the investigation of the granularity of the anti-PLA2R1 response in term of IgG1 versus IgG4 isotypes provided a potential new way to stratify the patients’ population and potentially tailored the therapy or follow the disease progression. Moreover, the isolation of patient’s derived anti-PLA2R1 antibodies opened the possibility of using them as tools to establish new models directly from the in vivo-selected specificities. Finally, the identification of an immunodominant epitope within PLA2R1 shared among the patients is crucial to develop molecules aimed at depletion of PLA2R1 specific memory B cells and plasma blasts in the patients.